4.1 Executive Summary

The Combined Highly Active Anti-Retroviral Microbicides (CHAARM) project carried out research aimed at developing novel microbicides from 1st January 2010 until 30th June 2015 with funding from the European Commission under the 7th Framework Programme. The CHAARM project involved 32 participating institutions from 9 countries of the European Union as well as from Switzerland, the Republic of South Africa and the United States. The consortium included 5 SMEs and 2 large industrial participants. A full list of partners can be seen from this link: http://chaarm.eu/content/partners.

The overall aim of the project was to develop new microbicides against HIV with a focus on developing microbicides that included combinations of anti-retroviral drugs and to investigate the potential for protease inhibitors to be used as microbicides. The project included drug discovery and development, a comprehensive microbicide testing platform, formulation studies including drug targeting as well as gels and intra-vaginal rings for delivery of microbicides to vaginal and rectal mucosae. In addition, studies of the vaginal microbiome were performed since the composition of the microbiome affects susceptibility to HIV infection. A feature of the programme was the facility to test microbicides in non-human primate (NHP) models for both pharmacokinetic properties and efficacy. The lead microbicide (a combination of 2 anti-retroviral drugs, Dapivirine and Darunavir) was tested in a phase I clinical trial.

Major outcomes of the project were the demonstration that the Dapivirine + Darunavir combination gel was safe in humans and the development of 3 further candidate microbicides through testing, formulation, pharmacokinetic and efficacy studies in NHP models. These are described in more detail in the following sections of this report. The project established a rigorous protocol for pre-clinical testing of microbicides both for efficacy and safety that allowed comparison of different candidates and informed decisions about candidates to progress further along the development pathway. Use of cervicovaginal and colorectal tissue explants provided more physiologically relevant models for testing of microbicides and were used extensively to test the effects of combining different drugs. Co-formulation of drugs in a single vehicle presented a particular challenge that was solved both by use of intravaginal rings and by inclusion of selected excipients in gel formulations. Extensive structural analyses of CCR5 interaction with inhibitors and of HIV integrase and reverse transcriptase enzymes provided a basis for development of more effective inhibitors of HIV replication both within the timeframe of the CHAARM project and beyond.

In addition to completion of the phase I trial, major impacts of the project were the development of a novel non-nucleoside inhibitor of HIV reverse transcriptase (NNRTI) – UAMC-01398. This NNRTI proved to be active in vitro against HIV strains resistant to other NNRTIs and showed significant protection in the NHP model. A protein-based microbicide, VHH J3, which has a different mechanism of inhibition from existing drugs was also developed through to NHP efficacy trials in the programme. Other impacts are described more fully in following sections.

Dissemination activities were led by Minerva Communicating and Consulting and by the European AIDS Treatment Group. In addition to publishing approximately 150 publications in scientific journals and approximately 200 presentations at various conferences, satellite workshops were organised at two of the annual conferences of the European AIDs clinical

society. Information about the programme was also disseminated through social media and the project website (chaarm.eu).

4.2 Summary description of project context and objectives

HIV/AIDS remains a major global health concern. Improved access to anti-retroviral drugs has resulted in a gradual decline in new infections. Nonetheless, the UNAIDS Global Report for 2014 estimates that in 2014, 2 million people were infected with HIV. There remains an urgent need for effective methods to prevent new infections.

The potential for topically applied inhibitors of HIV (termed "microbicides") to prevent infection has been investigated intensively over the last decade. The CHAARM project is a collaborative international programme that has contributed to this effort. Shortly after the start of the CHAARM programme, promising results were published from a phase IIb clinical trial (CAPRISA 004 study) in which application of a vaginal gel containing 1% Tenofovir (an inhibitor of HIV reverse transcriptase) conferred approximately 40% protection against infection. Towards the end of the CHAARM programme, a larger phase III trial of the same gel (FACTS 001 study) demonstrated no significant protection against infection. This disappointing result was attributed to very low levels of correct use (compliance) of the vaginal gel. Further analyses indicated that in women who used the gel frequently (confirmed by the presence of Tenofovir in blood) were significantly protected against infection. These findings indicate that, for various reasons, vaginal gels may only be used by a minority of women but that they can be effective when compliance is high. Thus other means of delivery are required so that choices can be offered. Use of slow release devices such as intravaginal rings loaded with anti-retroviral drugs or injection are two approaches under investigation.

The CHAARM programme aimed to develop new microbicides and had a particular emphasis on developing combinations of highly active specifically-targeted microbicides for vaginal and rectal application. Using two or more inhibitors of HIV activity in a single formulation may increase the effectiveness of a microbicide and is likely to decrease the likelihood that drug-resistant strains would infect or develop. After stringent testing *in vitro* for efficacy and safety, the most successful compounds were formulated and tested in macaque challenge models. As well as gel formulations, silicon elastomer intra-vaginal ring formulations were investigated for the candidate microbicides. As above, the latter may provide a more acceptable means of delivery of microbicides since once inserted rings will provide sustained release over a relatively long period. Inflammation at vaginal or rectal surfaces is associated with increased likelihood of infection with HIV. This condition may be linked to changes in the bacterial community (microbiome) found at these surfaces. It is clearly important that topical application of a microbicide does not perturb the microbiome associated with health. Studies of mucosal biomarkers were therefore included in the research programme to determine parameters associated with health and provide a basis for assessments of changes likely to be associated with mucosal damage. As indicated in the specific objectives listed below, a major objective was to perform a phase I safety trial of a combination microbicide.

Specific research objectives of the proposal were:

1. To investigate the potential of protease and proteasome inhibitors as microbicides.
2. To develop new small molecule inhibitors of HIV-1 reverse transcriptase and integrase.
3. To develop new small molecule inhibitors of HIV-1 fusion and further the development of novel protein and peptide based inhibitors as microbicides.
4. To test efficacy and safety of novel microbicide combinations using in vitro systems.

5. To develop procedures for co-formulation of microbicide combinations suitable for testing in vivo.
6. To test efficacy of selected microbicide combinations in macaque models of vaginal and rectal challenge.
7. To perform human studies including phase I trial of microbicide combinations.
8. To investigate mucosal biomarkers associated with mucosal health by analysis of microbiota, immune factors and the proteome of vaginal fluid.

4.3. Description of main S & T results

The main outcomes from the CHAARM programme were: i. completion of a phase I clinical trial of a combination microbicide (Dapivirine + Darunavir); ii. testing of selected compounds in non-human primate pharmacokinetic and challenge studies; iii. development of formulations to include combinations of anti-retroviral drugs (ARVs); iv. establishment of a comprehensive platform for testing microbicide efficacy and safety; v. development of novel candidate microbicides; vi. comparative analyses of the vaginal microbiome. These are described in more detail below.

4.3.1 Phase I clinical trial of a combination microbicide of Dapivirine and Darunavir

Responsible scientist: Professor Charles Lacey, University of York

To date, ARV microbicide development efforts have mainly focussed on non-nucleoside inhibitors of HIV reverse transcriptase (NNRTIs) especially Dapivirine as well as nucleotide inhibitors such as Tenofovir. Other ARVs include protease inhibitors (PIs), integrase inhibitors, and CCR5 blockers. Some of the characteristics of these agents are summarised below in the Table 1, below. Two relevant issues concerning ARV microbicides should be emphasised at this point: (a) genital tract administration of an ARV microbicide will not only result in substantial local drug concentrations of that ARV, but also in systemic absorption of small but detectable concentrations. (b) even very low concentrations of NNRTIs alone are sufficient to drive HIV resistance mutations in 7-14 days.

ARV class	Overall genetic barrier to resistance	Single mutation leads to high grade resistant virus	Class potency
NNRTIs	+/++	Yes (multiple)	+/++
NRTIs	+	Yes (Tenofovir – K65R)	++
PIs*	+++	No	++/+++
Integrase inhibitors	+	Yes	++
CCR5 blockers	++	No	++

*Protease inhibitors

Table 1. Genetic barrier to resistance for different classes of ARV.

Thus the development of ARV microbicides needs to be approached with careful thought. The concept of differing genetic barriers to the evolution of resistant virus with differing ARV classes and combinations is a constant issue for clinicians treating patients, but this has not been investigated extensively in the microbicide field. In general the genetic barrier to the development of resistance among ARV classes is NNRTIs < NRTIs < PIs. It seems logical therefore to consider protease inhibitors as ARV microbicide candidates because of their high genetic barrier to the evolution of resistance. Indeed boosted PIs such as Kaletra (Lopinavir + Ritonavir) are now being used as monotherapy. Such PI-monotherapy patients usually maintain

viral suppression and in those failing to suppress HIV viral load the evolution of PI-resistant virus is not usually seen.
It can be argued that protease inhibitors, unlike RT inhibitors, act post-viral integration and are not a logical choice as prophylactics. However macaque transmission studies have suggested that establishment of infection is dependent upon "broadcasting" of an initial isolated mucosal foci of infection to disseminating lymph nodes and beyond. PIs would be ideally suited to prevent such secondary "broadcasting" leading to an aborted infection.

With this rationale, a combination microbicide comprising Darunavir (PI) and Dapivirine (NNRTI) was formulated and tested *in vitro* as described below. Darunavir was also tested for safety in a rabbit vaginal tolerance study. A single centre, two-arm, double-blinded phase I randomised clinical trial was then performed. The primary objective of the trial was to assess the safety, pharmacokinetics and pharmacodynamics of Darunavir, and Darunavir + Dapivirine vaginal microbicides after a single dose and after a multiple dose regimen over a period of 2 weeks. The secondary objective was to assess the vaginal microbiome pre- and post-microbicide use since perturbations in the microbiome may promote inflammatory conditions. Each arm comprised 12 women. On completion of the trial it is evident that there is an excellent safety profile indicating that that both the Darunavir only and Darunavir-Dapivirine gels are safe. No changes in vaginal flora were observed over the trial period.

These findings, together with the comprehensive *in vitro* testing, formulation and pre-clinical toxicology studies (described below) provide a platform for further product development of this microbicide combination.

4.3.2 Testing of microbicides in non-human primate (NHP) pharmacokinetic and challenge studies

Responsible scientist: Professor Roger Le Grand, Commissariat à l'énergie atomique et aux énergies alternatives

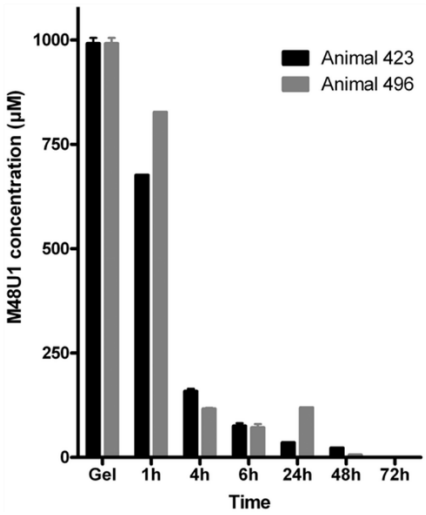
Several pharmacokinetic (PK) studies were performed in NHP models to assess in vivo distribution of drugs after mucosal application and to assess which formulations were optimal. In addition, PK studies provided data to inform the timing of HIV challenge after microbicide administration in experiments designed to test whether microbicides were effective in preventing infection. Three candidate microbicides showed efficacy in NHP challenge studies performed as part of the CHAARM programme, namely, a peptide mimic of the CD4 binding site for HIV gp120 (miniCD4), a single domain antibody fragment derived from a llama antibody (VHH J3) and a novel NNRTI (UAMC-01390).

MiniCD4 (Dereuddre-Bosquet et al. 2012, PLOS Pathogens 8 (12), e1003071)

In this study, PK studies were performed with the miniCD4 formulated in hydroxyethyl cellulose gel. As shown in Fig.1, at 1 hour after application, the level of miniCD4 as measured in vaginal fluid remained at about 75% of the initial concentration and was significantly reduced at later time-points although it should be pointed out that the levels of miniCD4 at 6 hours are still some 3-4 logs higher than the *in vitro* IC_{50} values for HIV neutralisation. Informed by these findings, a challenge experiment was performed in which macaques were challenged with chimaeric $SHIV_{162P3}$ in a single high dose challenge model at 1 hour after vaginal administration of the miniCD4 gel. In the control group which received placebo gel, 6 out of 6 animals became infected (Fig.2). In contrast in the experimental group, 5 out of 6

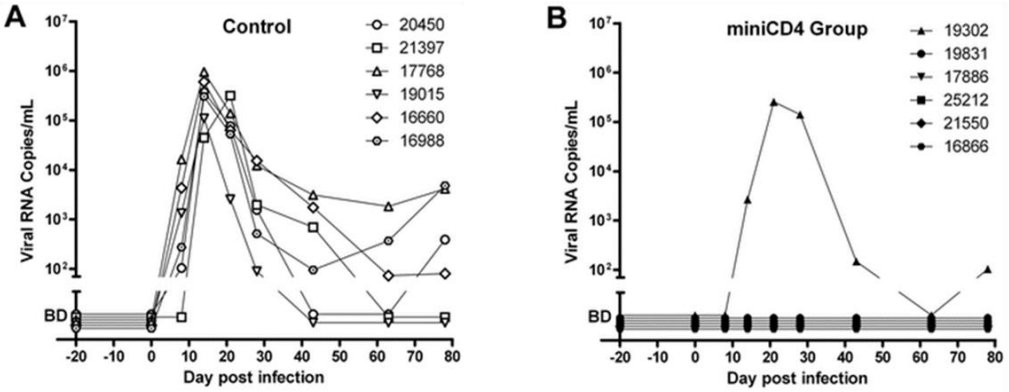
animals in the experimental group were fully protected. This result clearly demonstrated that the CD4 mimetic miniprotein can efficiently protect macaques from SHIV challenge, indicating that this small peptide, acting as a fusion entry inhibitor, could represent a new

Figure 1. Pharmacokinetic studies in non-infected macaques.



preventive agent against sexual transmission of HIV-1 when formulated as a microbicide. Because of its stably folded scaffold, this peptide possesses high stability and resistance to temperature and protease degradation. Contrary to other entry inhibitors, miniCD4 targets the virus and not a human receptor. Moreover, it is not used in current HIV therapy. This might provide a significant advantage as it may prevent the spread of viruses that become resistant to current treatments.

Figure 2. Protection of macaques against vaginal SHIV challenge by pretreatment with M48U1 gel.



VHH J3

Llamas (and other camelids) have both conventional antibodies comprising heavy and light chains but also have antibodies comprised of heavy chains only. The antigen binding domain of the latter retains high affinity binding in the absence of any light chain. Antibody fragments comprising the variable antigen-binding domain only, can be produced by microbial fermentation which allows low cost large scale production. In the CHAARM programme, a single domain antibody variable region (termed " VHH") has been produced (as described

below) that possesses highly potent neutralising activity against a wide range of HIV clades and strains. In unpublished experiments, this VHH has shown significant protection against infection in the same macaque challenge model as above.

UAMC-01398

A novel NNRTI (UAMC-01398) also developed in the CHAARM programme (as described below) was formulated in hydroxyethyl cellulose gel and tested for efficacy in a low dose repeated challenge macaque model of infection (Figure 3). Again, in unpublished work, UAMC-01398 showed significant protection.

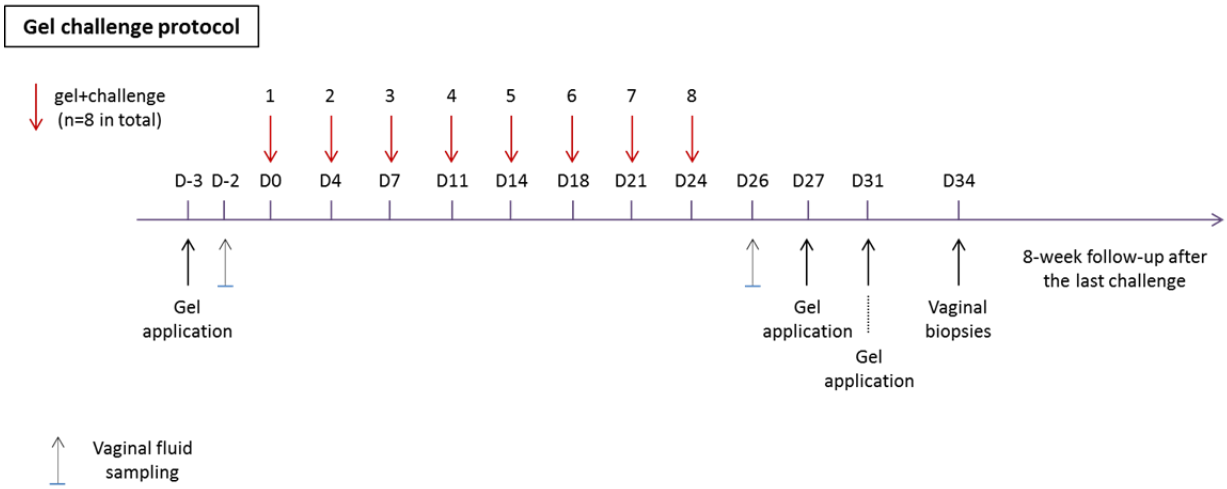


Figure 3. Protocol used for low dose repeated challenge with SHIV in NHP model of infection

PK studies

In addition to PK studies linked to challenge experiments, other studies were carried out with the gel and intravaginal ring formulations of the Dapivirine + Darunavir combination. The data shown in Fig. 4 show that sustained release of both drugs over a period of at least 30 days is obtained with the intra-vaginal ring. Dapivirine and Darunavir were delivered to the vaginal region at concentrations over 4 logs and 3 logs greater than the reported EC50 for wild-type HIV-1 (LAI), respectively (EC_{50}LAI of dapivirine: 0.3 ng/ml - EC_{50}LAI of darunavir: 1.6 ng/ml). With the gel co-formulation (identical to that tested in the phase I clinical trial, as above), drug concentrations are initially higher than those obtained with DAP/DRV rings and remain high 72 hours after dosing. Thus both formulations may provide protection for a significant time following application.

4.3.3 Development of formulations to include combinations of anti-retroviral drugs (ARVs)

Responsible scientists: Dr Mark Mitchnick, Particle Sciences Inc; Professor Karl Malcom, Queen's University Belfast; Professor Patrick Augustijns , Katholieke Universiteit Leuven; Dr Jens Van Roey, Janssen Diagnostics

As discussed briefly above, both gel and vaginal ring formulations were investigated in the CHAARM programme. It is not expected that any single formulation is likely to be acceptable

by all users and therefore there may be advantages in developing multiple formulations. Co-formulation of Dapivirine and Darunavir in a single gel proved unexpectedly problematic in that on combining the drugs, a small but significant proportion of Darunavir undergoes a degradation reaction. After considerable further work (and with additional funding provided by Janssen Diagnostics), this degradation was mitigated to an acceptable extent by inclusion of specific excipients in the gel formulation that was used in the phase I trial and macaque PK studies.

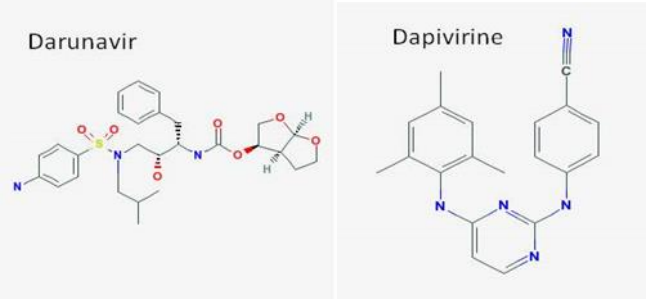


Figure 4. Structural formulae of Darunavir and Dapivirine

Silicone elastomer rings in which both drugs were incorporated into the matrix were produced and showed favourable release characteristics in macaque PK studies, as above (Murphy et al. 2014. J Antimicrob Chemothher 69: 2477-88). Dapivirine only rings are currently being assessed as microbicides 2 linked phase III clinical trials (the Ring Study and ASPIRE). Results from these trials may indicate whether there is room for investigation of combination microbicides to improve efficacy.

4.3.4 Establishment of a comprehensive platform for testing microbicide efficacy and safety

Responsible scientists: Professor Guido Vanham, Institute for Tropical Medicine Antwerp; Professor Elisa Vicenzi, Fondazione Centro San Raffaele; Professor Robin Shattock, Imperial College London

An important aspect of microbicide development in CHAARM was the establishment of an independent platform for testing HIV neutralising activity in cell and tissue explant model systems. Cytotoxicity was also determined to provide selective therapeutic index. Several hundred candidate molecules were tested in this platform with many showing insufficient potency but providing valuable structure activity data and/or undergoing further reiterative development. The most promising candidates were then tested for safety using a whole blood assay. Following incubation with candidate microbicides, release of pro- and anti-inflammatory cytokines in whole blood was measured using a panel that included IL-6, IL-8, IL-10 and MCP-1.

Reflecting the emphasis on developing combination microbicides, several combinations of microbicide were investigated *in vitro* to determine whether efficacy was improved. A number of combinations demonstrated at least additive and in some cases synergistic effects (Ferir et al. 2011. Virology 417:253-8; Ferir et al. 2012. Virology 433: 308-19; Ferir et al. 2012. AIDS Res Hum Retrovir 28: 1513-23; Secchi et al. 2014. Antimicrob Agents Chemother 58:6215-23).

A further aim of this microbicide testing platform was to investigate the development of resistant viruses by stringent *in vitro* selection procedures so as to determine mechanisms of resistance. Of particular interest, the NNRTI, UAMC-01398, demonstrated a high genetic barrier to the development of resistance. Moreover, resistant strains showed reduced viral fitness compared with wild type strains.

4.3.5 Development of novel candidate microbicides

Responsible Scientists: Professor Koen Augustyns, University of Antwerp; Professor Robin Weiss, University College London; Professor Theo Verrips, University of Utrecht

In this section, development of the candidate microbicides that progressed to challenge studies in the NHP model will be emphasised. Several other participants in CHAARM performed outstanding research to develop potential candidates that block co-receptor binding, HIV integrase as well as formation and nuclear import of the integrase complex. These studies required extensive molecular structure analyses and have provided a significant for future development of HIV inhibitors. The significance of this work is indicated by a series of articles in high impact journals.

UAMC-01398

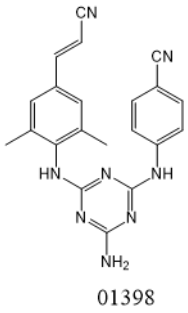


Figure 5. Structural formula of UMAC-01398.

With Dapivirine as a starting point for design of a novel NNRTI and after rounds of testing for neutralising activity against HIV, the cyanovinyltriazine UAMC-01398 (Fig. 5) demonstrated potent HIV neutralising activity and low cytotoxicity as shown in Table 2, below. As part of the development, synthesis of the compound had to be modified such that one of two isomers was the only product (Venkatraj et al. 2012. Bioorg Med Chem Lett 22:7174-8; Arien et al. 2013. J Antimicrob Chemother 68:2038-47).

UAMC / Ref	Wild type EC_{50} (nM)		NNRTI- resistant viruses EC_{50} (nM)					Cytotoxicity CC_{50} (nM)
	TZM-bl		Ba-L V106A (NVP)	VI829 Y181C (NVP)	VI829 L100I+K103N (EFV)	Ba-L * (TMC120)	VI829 ** (TMC120)	
	Ba-L	VI829						
01398	1.3	1.3	1.9	2.6	6.8	23.0	89.0	24540
Dapivirine	2.0	2.0	2.5	10.8	673.5	> 1000	> 1000	2877

Table 2. Neutralising activity of UAMC-01398 against wild type and resistant strains of HIV compared with Dapivirine.

As shown in Table 2, UAMC-01398 show comparable neutralising activity against wild type and nevaripine-resistant strains to Dapivirine. UAMC-01398 was considerably more effective against efavirenz- resistant and Dapivirine (also termed TMC120)-resistant strains. UAMC-01398 was formulated in a hydroxyethylcellulose gel (Grammen et al. 2014. Antiviral Res 101:113-21) and as indicate above, was subsequently tested in a macaque challenge experiment.

VHH J3

The llama-derived single domain VHH J3 was isolated from a phage display library by high throughput screening for HIV neutralising activity (McCoy et al. 2012. J Exp Med 209:1091-1103). As shown in Fig. 6, J3 possesses potent neutralising activity against a wide range of HIV strains.

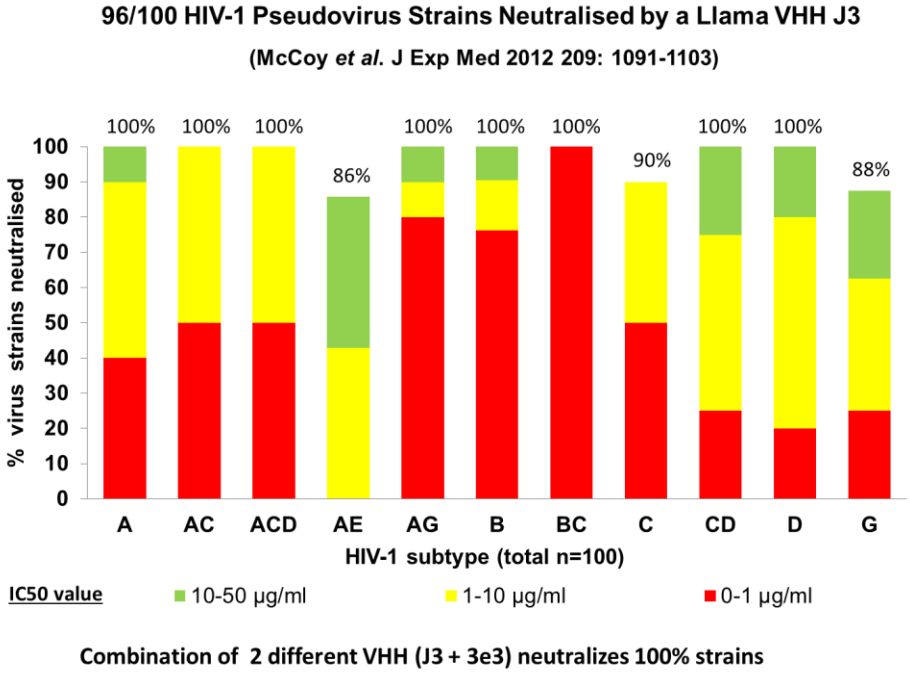


Figure 6. Neutralising activity of the llama VHH J3 against HIV pseudovirus strains

The VHH was formulated in hydroxyethylcellulose gel and also tested in a macaque challenge experiment as described above.

4.3.6 Comparative analyses of the vaginal microbiome

Responsible scientists: Dr Vicky Jespers, Institute for Tropical Medicine Antwerp; Professor Janneke van de Wijgert, University of Liverpool

Studies of the vaginal microbiome performed by CHAARM participants and in collaboration with EDCTP funded projects (Kyongo et al. 2012. PLos ONE 7(8) e43951; Gautam et al. 2015. BMC Inf Dis 15(86)1-14; van de Wijgert et al. PLoS ONE 9(8):e105998) contribute to and support the consensus that lactobacilli-dominated vaginal microbiota are associated with a healthy vaginal micro-environment. Dominance by *Lactobacillus crispatus* was associated with a low diversity microbiome and in a study of sex workers in Rwanda (Borgdorff et al. 2014. ISME Journal 8:1781-93) was associated with reduced HIV and sexually transmitted infections. At the other end of the spectrum, microbiota where diversity was high and

dominated by anaerobic bacteria such as *Gardnerella vaginalis* and *Atopobium vaginae*, were associated with bacterial vaginosis and increased susceptibility to HIV infection. Microbiota dominated by *Lactobacillus iners* showed increased diversity compared by those dominated by *L. crispatus* and a small increase in susceptibility to HIV infection. Thus *L. iners* may be less beneficial than *L. crispatus*.

These studies show that bacterial vaginosis (BV) is best described as a polybacterial dysbiosis. The extent of dysbiosis correlates well with Nugent score and vaginal pH but not with the other Amsel criteria. In addition, longitudinal studies have shown that a *L. crispatus*-dominated microbiota is more likely to shift to a *L. iners*-dominated or mixed lactobacilli microbiota than to full dysbiosis. In contrast, a *L. iners*-dominated vaginal microbiota compared to one dominated by *L. crispatus* is twice as likely to transition to a bacterial vaginosis-associated microbiota.

4.4. Potential impact, main dissemination activities and exploitation of results

Impact

The UNAIDS Global Report for 2014 (issued in 2015) estimates that in 2014, 2 million people were infected with HIV. An effective means of prevention such as microbicides therefore remains an urgent need. The lack of protection observed in the FACTS 001 phase III trial of 1% Tenofovir gel can be attributed to poor compliance with use of the gel. Estimates of compliance (supported by detection of drug at quarterly visits of trial participants) indicated that the gel was used in only 50-60% of sex acts. That the product could be effective when compliance was high was evident from nested case-control evaluations of women with detectable drug levels comparedto those without. Protection of 52% was observed in this group which represented approximately 20% of participants. These findings point to the need to consider what means of microbicide delivery will improve levels of compliance. Phase III trials of Dapivirine formulated in a vaginal ring, currently in progress, may inform future development.

The extensive pre-clinical and Phase I studies performed during the CHAARM programme has resulted in Janssen (a CHAARM participant) having active discussions with a development partner to further the Dapivirine-Darunavir combination or other combinations in the prevention of sexual transmission of HIV. These could involve ring formulations as developed in the CHAARM programme.

The CHAARM programme has also contributed to the pipeline of candidate microbicides by developing novel compounds that may be effective against strains of virus that are resistant to current drugs. Increasing the efficacy of microbicides will clearly impact significantly in further reducing the number of infections globally. More specifically, research in the CHAARM programme has had impacts as anticipated in the Description of Work.

i. Increase knowledge of basic biological processes of disease and facilitate European research in the area

The use of *in vitro* models of infection such as dendritic cell co-culture and cervical and rectal tissue explants to assess microbicide efficacy were further developed by members of the

CHAARM consortium. These models provided a means for testing microbicides in more physiologically relevant systems.

Studies of the basic cell biology of HIV infection by researchers in the consortium has identified new targets for intervention. Zeger Debyser and colleagues at K.U.leuven had previously identified lens epithelium–derived growth factor (LEDGF/p75) as a cellular cofactor of HIV-1 integrase (IN) that interacts with IN through its IN binding domain (IBD) and tethers the viral pre-integration complex to the host cell chromatin. In a newer study, they reported generation of a LEDGF/p75 knock-out cell line in which HIV replication is considerably reduced. The residual replication was predominantly mediated by the Hepatoma-derived growth factor related protein 2 (HRP-2), the only cellular protein besides LEDGF/p75 that contains an IBD. Importantly, the recently described IN-LEDGF/p75 inhibitors (LEDGINs) remained active even in the absence of LEDGF/p75 by blocking the interaction with the IBD of HRP-2 (Schrijvers et al. 2012. PloS Pathogens 8:e1002558). In a further study, direct binding of the nuclear import factor transportin 3 to the integrase tetramer in the intasome complex was demonstrated (Larue et al. 2012. J Biol Chem 287:34044-58).

Maurizio Botta and colleagues at the University of Siena investigated inhibitors of hematopoietic cell kinase (Hck) which is a member of the Src family of non-receptor protein tyrosine kinases (SFKs), the function of which comprises various signaling pathways involved in the regulation of several processes (Tintori et al. 2013. ChemMedChem 8:1353-1360). Hck is involved in immune signaling and cell proliferation in hematopoietic cells and is linked to cancer. Furthermore, Hck activity has been associated with viral infections including HIV-1. In particular, Hck is activated by the HIV-1 accessory protein negative regulatory factor Nef, a multifunctional HIV-1 protein that accelerates progression to acquired immune deficiency syndrome (AIDS) and enhances the infectivity of progeny viruses. In this study the authors demonstrate inhibition of HIV replication with one inhibitor of HcK confirming this kinase as a target for intervention.

Similarly Jose Alcami (Instituto de Salud Carlos III) and colleagues demonstrated that specific inhibitors of protein kinase cθ inhibited HIV replication by reducing T cell activation (Lopez-Huertas et al. 2011. J Biol Chem 286:27363-77). Other examples of identification of novel targets that define requirements for interaction with cellular co-factors to support HIV replication are included in the list of publications resulting from research in this programme.

ii. Support discovery and development of more efficient microbicides against HIV/AIDS

Development of protease inhibitor-based microbicides was a novel aspect of the CHAARM programme. In vitro studies confirmed the efficacy of such inhibitors in cellular and tissue explant models. Moreover, a phase I clinical trial of Darunavir alone together with a combination of Darunavir + Dapivirine was completed allowing further development of this microbicide candidate. If the concept is valid, the distinct mechanism of microbicidal action offered by protease inhibitors will be useful in developing combination microbicides with increased breadth of coverage against divergent HIV-1 strains, reduced probability of transmitting viruses resistant to any single inhibitor, and possible microbicide synergy, creating dose-sparing effects.

Improved models to measure *in vitro* release of microbicides were also developed as proposed. These included a dual chamber model of vaginal epithelium using the HEC-1A cell line to form

a barrier epithelium (Gali et al. 2010. J Virol Methods 165:186-97; Gali et al. 2010. Antimicrob Agents Chemother 54:5105-14; Grammen et al. 2012. Antiviral Res 96: 226-33). In addition, extensive PK studies in macaques (as indicated in section 1.30 provided valuable *in vivo* data on drug release from formulations and most recently,novel data on release of Darunavir has been provided by the phase I trial conducted in the CHAARM programme. These data have been and will be disseminated by publication in scientific journals.

iii. Strengthen European competitiveness in this area

A strong ethos of collaborative research was necessary in the consortium so as to progress candidate microbicides through testing in various models to formulation and PK studies and in a limited number of cases to efficacy trials in the NHP model. During the course of the CHAARM consortium, two further projects involving CHAARM participants (as well as new participants) were funded under Framework 7, namely, MOTIF and AIM-HIV.

iv. Integration of expertise from different disciplines

Disciplines ranging from structural biology, synthetic chemistry, phage display technology, virology, physiology, immunology, biochemistry, microbial genetics, non-human primate studies, proteomics and clinical science ("from crystallography to the clinic") were all represented in this consortium and contributed to the development of microbicide candidates. Particularly strong structural biology research underpinned development of improved CCR5 inhibitors and integrase inhibitors as well as providing data on the mode of action of the single domain VHH J3 fusion inhibitor.

v. Formation of partnerships between public and private institutions

An important aspect of the CHAARM programme was the involvement of industrial participants (Janssen Diagnostics, Gilead and Particle Sciences). Indeed, as indicated above Janssen is in discussions about further development of Dapivirine + Darunavir as a combination microbicide.

vi. Involvement of research groups from developing countries

The consortium included one beneficiary based in South Africa at the CSIR, Pretoria. This is of course a well-resourced institution that produces high quality science. However the transfer of technology from the University of Geneva for the production of clinical grade RANTES analogues for future microbicide development was of mutual benefit.

Dissemination activities and exploitation of results

Dissemination activities have been led by CHAARM consortium members Minerva (based in Brussels) and the European AIDS Treatment Group (EATG).

Approximately 150 papers have been published in peer-reviewed scientific journals with several more expected to be submitted in the future. Researchers have presented project findings at the international Microbicides conferences in 2010 and 2012 as well as the HIV Research for Prevention conference held in 2014 (total of 21 presentations) and at the annual Conferences on Retroviruses and Opportunistic Infections (13 in total) as well as making approximately 120 oral or poster presentations at a variety of national and international conferences.

More widely, project progress and events were disseminated by means of the project website and by social media. Involvement of EATG as a dissemination partner facilitated dissemination to stakeholders. A video including interviews with CHAARM participants was produced as a dissemination tool and can be accessed by the link below:

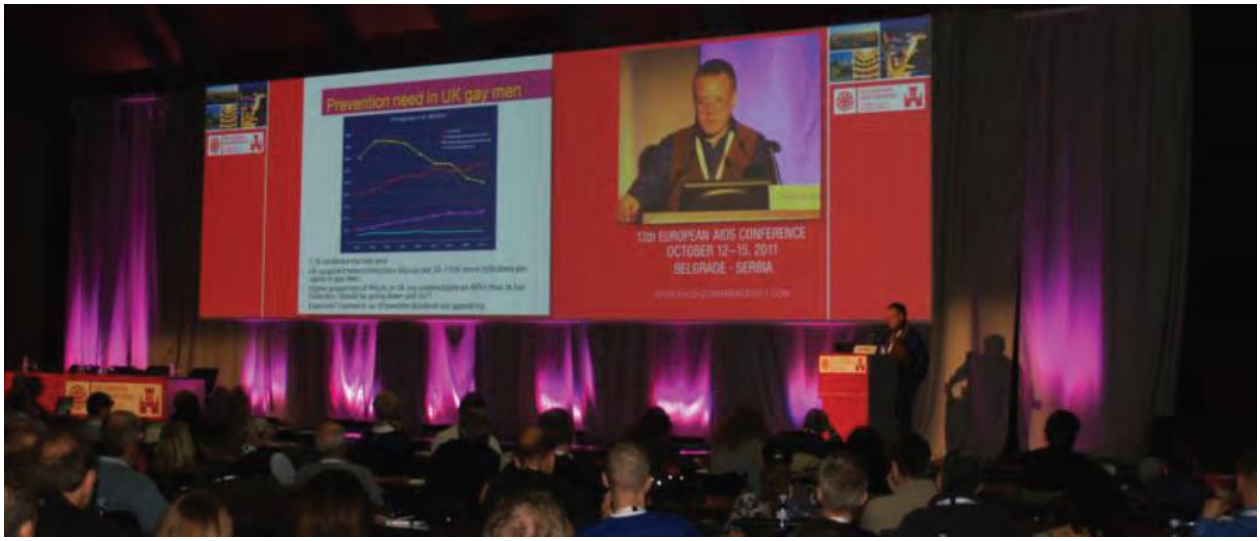
https://www.youtube.com/watch?v=ia_hv4vZ6SU

Annual newsletters were produced and can be accessed by the link:

http://chaarm.eu/newsroom/newsletters

During the project, two satellite symposia were organised to be held in conjunction with the European AIDS Clinical Society conferences.

The first was held in Belgrade on 12th November 2011 and covered the topic "Advances in HIV biomedical prevention research: Why involving the Community is key."
Speakers were: Charles Kelly, Charles Lacey, Christiana Noestlinger, François Berdougo and Gus Cairns.



Gus Cairns at EACS satellite symposium, Belgrade

The second satellite was held in Brussels on 16th October 2013 and covered the topic "New HIV Prevention Strategies: the future of Microbicides"
Speakers were: Sheena McCormack
Guido Vanham
Janneke van de Wijgert
Harriet Langanke
Alessandra Martini
Gus Cairns

From left to right: Alessandra Martini (European Commission), Harriet Langanke (Gemeinnützige Stiftung Sexualität und Gesundheit), Janneke van de Wijgert (University of Liverpool), Guido Vanham (University of Antwerp) and Sheena McCormack (Imperial College London)

4.5. Address of project website:

chaarm.eu